From DEPARTMENT OF NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

HOME-CAGE MONITORING OF MOUSE BEHAVIOURS ACROSS LIFE-SPAN

Karin Pernold

Stockholm 2019

The cover image, two heat maps illustrates the switch in activity pattern of female mice when they are introduced to dietary restriction (heat map to the right) compared to their controls (heat map to the left)

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by E-PRINT
© Karin Pernold, 2019
ISBN 978-91-7831-428-7

Home-cage monitoring of mouse behaviors across life-span
THESIS FOR LICENTIATE DEGREE

By

Karin Pernold

Principal Supervisor:
Professor Brun Ulfhake
Karolinska Institutet
Department of Neuroscience

Co-supervisor(s):
Velmurugesan Arulampalam, PhD
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Associate Professor, Rochellys Diaz Heijtz
Karolinska Institutet
Department of Neuroscience

Examination Board:
Professor Abdul K H Mohammed
Linnaeus University
Department of Psychology

Docent Erika Roman
Uppsala University
Department of Pharmaceutical Biosciences

Professor Klas Blomgren
Karolinska Institutet
Department of Women's and Children's Health

ABSTRACT

Background and aim

The academic research involving animal testing is struggling with problems of reproducibility and a few of the specific reasons pointed out are for example insufficient reporting of animal strains and protocol details. It is everyone's responsibility to follow the 3R's (Replace, Reduce, Refine) and to ensure that the model used is well characterized.

In this work we investigated how activity data collected from automated home-cage monitoring can be used to characterize behavior patterns and how well the results could be reproduced in a multi-center setup. We also studied if we can detect changes in behavior patterns through aging, using the same system. Finally, the impact of dietary restriction on behavior patterns and activity levels.

Material and Methods

We used C57BL/6J male and female mice from young age and kept them in the study for as long as up to 70 weeks. Groups of both male and female mice were subjected to modest dietary restriction from the age of 3 months and throughout the study.

The DVC ™ system uses standard IVC cages and has an external board of sensor electrodes in each cage slot that can detect activity on the cage floor in cages of group-held mice.

Results and conclusion

By analyzing activity data from the DVC system we could identify daily activity patterns with increased activity during lights off and also around the time for lights on in the holding room. We could also document the impact of husbandry procedures such as cage change. In addition, we identified additional behavioral rhythms with weekly variations and, importantly, a seasonal-like oscillation in activity with highs and lows and a periodicity of about 40 days.

We hypothesized that activity levels would decrease with increasing age, and there is a small but highly significant decrease in overall activity between young adulthood and middle age. Monitoring mice on dietary restriction, we show that the diet regime is able to completely change the activity patterns, but we were not able to detect clear differences in activity levels between dietary restricted and ad libitum fed mice.

LIST OF SCIENTIFIC PAPERS

I. Pernold K, Iannello F, Low B E, Rigamonti, M, Rosati G, Scavitzzi F, Wang J, Raspa M, Wiles M V, Ulfhake B. 2019. Towards large scale automated cage monitoring – Diurnal rhythm and impact of interventions on in-cage activity of C57BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique. PLoSONE 14(2): e0211063

II. Pernold K, Rullman E, Ulfhake B. Alterations in mouse home-cage behaviors during aging and in response to dietary restriction (manuscript in preparation)

CONTENTS

LIST OF ABBREVIATIONS

DR	Dietary restriction
CR	Caloric restriction
IPGTT	Intra-peritoneal glucose tolerance test
DVC	Digital Individually Ventilated Cage
INCA	Indirect Calorimetry
RQ	Respiratory Quotient
i.p.	Intra-peritoneal

1 INTRODUCTION

The mouse is one of the most commonly used animals in biomedical research. One advantage with using animals, including mice in experimental research is that they represent complete and also complex living organisms. The large number of different mouse strains including the genetically modified sub-strains offers great opportunities to choose or create a suitable model but in the name of the 3R's (Replace, Reduce and Refine) [1] it also includes a responsibility to understand the needs and characteristics of the specific model. Concerns have been raised towards the lack of reproducibility within academic research [2-4] and international initiatives, for example the International Mouse Phenotyping Consortium (IMPC) was created in order to standardize phenotyping procedures and increase knowledge about specific models. Despite the efforts, there are still problems and the specific critique concerns insufficient or incorrect reporting of mouse strains, husbandry, protocol details and lack of statistical power.

Systems for automated home-cage monitoring [5-13] started to develop and attracts more and more interest. Several different systems are available and with such systems, large amounts of data can be collected from the home-cage environment about animal's behaviors and activity without disrupting or disturbing them. If we can learn more about their basic activity and behavior patterns, we can better understand changes and abnormalities in these patterns. In the end, this could contribute to better husbandry protocols and improved reproducibility.

Aging is inevitable and many has taken on the challenge to define and explain this process, philosophers as well as biologists. The programmed theories of aging [14-15] suggests that aging is programmed in our genome and that there is a biological timetable. This theory finds support in the fact that in very different species it is possible to identify distinct episodes of the life-span (for example infanthood, puberty, adulthood, reproductive senescence of the mouse) that must be imprinted and that external, environmental cues will trigger these episodes. The opposing Disposable soma theory [16] about DNA damage and error states that repair must be good enough to ensure reproduction of the species [16] and in this way the accumulation of non-repaired damage would be a measure of aging. It has been shown that caloric or dietary restriction can prolong life-span and also health span [17].

2 MATERIAL AND METHODS

Material

Mice

Mice, C57BL/6J male and female, delivered from either Jackson Laboratories (Bar Harbour, US), Charles River (Germany) or Janvier (France), arrived aged 6-8 weeks and were kept 4 or 5 per cage in GR500 IVC cages (Tecniplast) with aspen or corncob bedding, cage enrichment and food and water ad libitum (Table 1).

Cohort		Home-cage monitoring in DVC	Husbandry	Additional procedures
40 x C57BL/6J males	Charles River, Germany 5 males/ cage	4+4 cages weekly and bi-weekly cage change for 10 weeks 4 of the above cages remained until age 15 months	100g aspen bedding, red plastic mouse house, sizzle nest Ad libitum food (SDS RM3 irradiated pellets) & water (weakly chlorinated, changed every week) Weekly cage change on fixed day 12/12hour light cycle Room temperature 19-23°C. Air humidity 40-60%. Lights on between 50-300 Lux.	NA
32 x C57BL/6J females	Charles River, Germany 4 females/ cage	3+3 cages From age 2 to 15 months Dietary restricted and ad libitum fed controls		Basal metabolism (INCA), body composition, estimated daily food intake, glucose tolerance test (only males)
40 x C57BL/6JRj males	Janvier Labs, France 4 males/ cage	3+3 cages From age 2 to 22 months Dietary restricted and ad libitum fed controls		
25 x C57BL/6J females	Jackson Laboratory, Maine, US 5 females/ cage	Multi-center (additional 25+25 mice at CNR and JAX) 5+5+5 cages From age 3 to 6 months	As above, but with 200g CornCob and a nestlet pad instead of sizzle nest	Additional, weekly BW on fixed day

Table 1 Summary of mouse cohorts, husbandry and experiments

The first cohort (Table 1) of 40 males was initially divided into two groups (4+4 cages with 5 males in each cage), subjected to weekly and bi-weekly cage change including body weight measuring for a period of 10 weeks. 4 of these cages were then used for continuous monitoring of home-cage activity until the age of 15 months.

The following two cohorts of 32 females and 40 males (Table 1) were subjected to the dietary restriction study and kept four mice per cage. The females remained in the study until age 15 months and the males until age 22 months.

The last cohort of females (Table 1) was part of a multi-center study conducted at three sites (Karolinska Institutet in Sweden, CNR in Italy and Jackson Laboratories in US). Each of the sites used 50 females each, bred from the same colony at Jackson Laboratories (Bar Harbour, US) and the three experiments was conducted with a few week's difference in time. These groups were weighed twice a week on fixed days, at cage change and on day 4 in the cage change cycle.

Methods

DVC and activation metrics

The DVC system is a digital IVC rack for mouse cages, where a board of sensing electrodes is positioned underneath each cage slot. The board is divided into 12 separate electrodes and a proximity sensor measures the electrical capacitance of each of the 12 electrodes, every 250 milliseconds, and can detect changes in the electrical capacitance produced by the dielectric properties of matters in close proximity to the sensors (i.e. mice or other objects in the cage). By analyzing the changes in electrical capacitance over the different sensors, movements in the cage can be detected.

Here we use activations per minute as a metric for activity. Briefly, activations are calculated as the difference in electrical capacitance from one measurement to the next (250 milliseconds) and compared to a threshold [13].

Dietary restriction

At age 12-13 weeks, when the mice are through puberty and young adults, 3 cages of females and 3 cages of males was randomly selected to dietary restriction (DR). All food in the food tray was removed and instead each cage was fed a pre-weighed amount once a day during lights on period (between 10am – 2pm). Females received 2.0g/mouse/day and males 2.5g/mouse/day which is approximately 70% of the amount that the ad libitum (AL) fed controls eat. Both DR and AL groups were weighed every week (at cage change) and at the same time, remaining food in the trays of the AL group were also weighed and re-filled with a fixed amount. Mean body weight for each group and each week was calculated and the AL

mice daily food intake per mouse in the AL group was estimated based on the cages weekly consumption.

Indirect Calorimetry

Basal metabolism was measured by indirect calorimetry apparatus (INCA; Somedic, Sweden) before and after introducing dietary restriction (males and females) as well as in older age (males, approximately 22 months old).

INCA system is described in detail [18-19], and briefly presented here. It compares measured levels CO_2 and O_2 (ppm or %) in air entering and exiting the test chamber every 2nd minute and calculates CO_2 produced and O_2 consumed as ml/ [min*kg] and respiratory quotient (RQ; the ratio of CO_2 production to O_2 consumption) as percent.

On the day of experiment, mice were brought to the experiment room and allowed to acclimatize to the room and the test cages (containing fresh aspen bedding, new sizzle nest and clean, red, plastic mouse house) for approximately 2 hours followed by another 2 hours of acclimatization inside the test chambers. During the experiment, which ran for 20 hours, mice were single housed and had ad libitum access to food and water. DR mice were fed their daily ration at start of first acclimatization, approximately 4 hours before start of experiment. Mean values and standard error of the mean (SEM) was calculated for each of the parameters O_2 consumption, CO_2 production and RQ, separate for lights off period (12 hours) and the total lights on period (8 hours).

Body Composition

Fat and lean body mass was estimated using EchoMRI (EchoMRI LLC, Texas, USA) in awake mice, before and after introducing dietary restriction (males and females) as well as in older age (males, approximately 22 months old).

Intra-Peritoneal Glucose Tolerance Test (PGTT)

Blood glucose clearance was tested with Intra-peritoneal glucose tolerance test in males (AL and DR) at older age, approximately 22 months old.

Mice were fasted for 4-5 hours in their home cage (from 7am to ~12pm) with access to water, while mice on DR were fasted since the previous feeding, 20-22 hours. A small cut was made on the tip of the tail, after treating it with Lidocain/Priolocain cream (EMLA™, 25mg/g). A few drops of blood was collected from the small cut, to measure baseline level of blood glucose, mmol/ml (using Accu-Chek blood glucose meter). Immediately after, glucose was

injected i.p. (2g/kg) and at 15, 30, 60 and 120 minutes, blood glucose was again measured in blood freshly sampled from the same cut.

Data analysis

All calculations on activations data collected from the DVC were performed using scripts in either Python, Mathlab or R version 3.5.0

Global activity was calculated as mean of activations over the 12 sensors per minute.

When analyzing activity levels across aging, minute aggregated global activations was averaged over 24 hours or weeks, separate for lights off (12 hours), lights on (12 hours) or weeks. A week starts on the day of cage change, at the time for lights on and ends at the day of the following cage change at lights on, i.e. 7 days.

Response to cage change, response to lights on as well as activity during lights off was analyzed using minute aggregated global activations from each event, averaged across cages and a number of weeks and/or days. A moving average of 30 or 45 minutes was used as a smoothening filter and from the smoothened data peak response, average response activity as well as response duration (FWHM) was calculated.

We used functional data analysis with R including linear regression of untransformed or transformed data and Fourier series for periodic data and functions. Activity-data (aggregated minute activations across all sensors of the board) from each cage divided into night- and day-time observations was centered and scaled to a mean of 0 with standard-deviation as principal unit where 1 is activity 1 standard-deviation above the average activity throughout the observation period. To account for the small but highly consistent decline in activity over time and make the time-series more stationary, a de-trending linear model was fitted for each time-series where after stationarity was confirmed using Augmented Dickey-Fuller Test where a p-value of <0.01 was considered a stationary time-series. Prior the analysis, each time-series was smoothed using loess-regression with a conservatively chosen span of 5% in order to maintain local peaks and variation. The mean and standard deviation of the distance in time between peaks in activity with an amplitude ≥ 1 SD and a minimal wavelength/seasonality of 2 weeks was calculated for each time-series of night-time recordings while analysis stringency was relaxed somewhat due to the generally lower level of activity of day-time recordings. In the functional analysis of rhythmicity all data were used and with minute resolution of the aggregated activations.

Statistics

To test differences in activity levels or response levels across cohorts, gender, age, weeks and days, rank-based analysis of variance-type statistics (AST) was done using the nparLD package in R. Cages was considered as subjects, weeks and weekdays (days in the cage change cycle) as within-subject factors, site, diet and sex as between-subject factors. This is a non-parametric test for longitudinal data that does not require as strong assumptions as the Repeated Measures ANOVA.

3 RESULTS

Characteristics of ad libitum fed (AL) and dietary restricted (DR) mice

Body weight and body composition

Dietary restriction (DR) was introduced around the age of 13 weeks. Previous studies [20, 21] has shown that this restriction extends life-span by around 20% in mice and rats and it also reduces variability in body weight between cage mates. As shown in figure 1, the lean body mass (LBM) as percent of total mass is not different between AL fed and DR mice and not different across time, but fat content is up to 3 times higher compared to DR mice. In late-life, fat content is reduced.

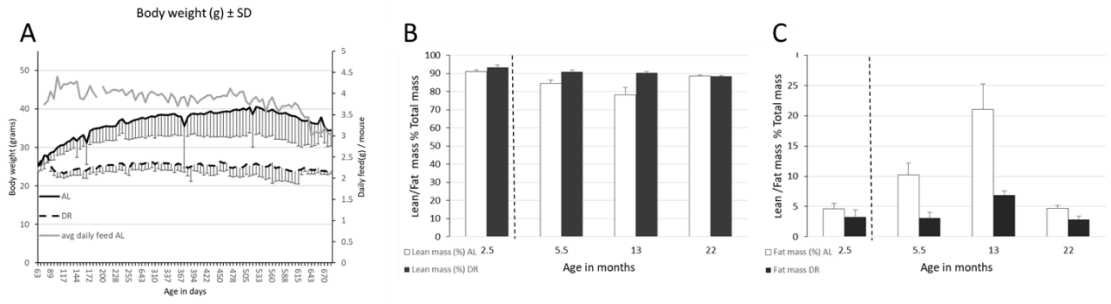

Fig. 1 (A) Weight curve male mice ad libitum fed (AL, continuous line) and dietary restricted (DR, dotted line) and estimated, daily food intake per mouse (grey line). When dietary restriction is introduced, this group initially lose weight and will then stabilize and not gain weight as the AL fed mice do. (B) Mean lean mass as percent of total mass and (C) fat mass as percent of total mass. White bars representing AL mice and black bars representing DR mice at ages 2.5 months (before introducing DR, indicated by a dotted line), 5.5, 13 and 22 months.

Indirect calorimetry

Basal metabolism was evaluated in both males and females before dietary restriction was introduced. Both groups displayed mean RQ values above 90% both during dark and light period in INCA test (Table 2). When measured again at age 12 months, RQ values averaged around 85% except the DR group which was lower (81%) during the dark phase, when they are normally at rest. Males, tested at age 5 months averaged at RQ 88% during the active phase (which is dark for AL group and light for DR group) and 84-85% during the less active phase.

The oldest group tested, 22 months old males all displayed RQ values between 85-89%, lowest was the DR group during dark phase.

Young females displayed the highest values (>100 ml/[min*kg]) O2cons and CO2prod during dark phase.

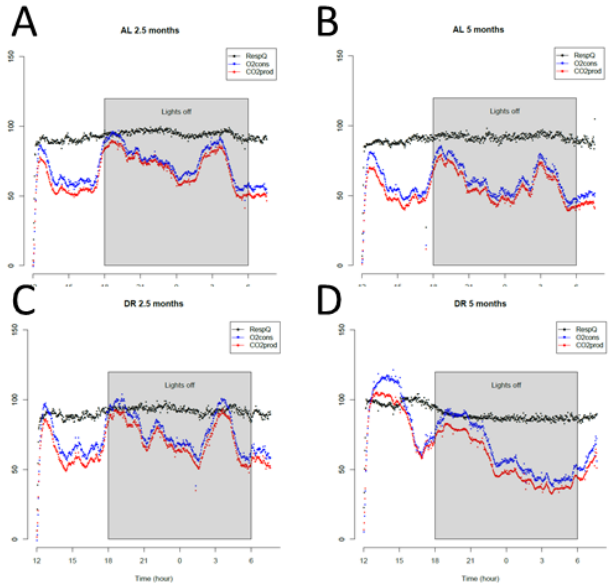

Fig 2. Basal metabolism of male mice aged 2.5 months (A and C), before introducing dietary restriction, repeated at age 5 months in ad libitum fed mice (B) and dietary restricted (D). Blue line represents O2 consumption in ml/(min*kg), the red line CO2 in ml/(min*kg) and the black line is the respiratory quotient (RQ) in percent. See also Table 2.

Levels of O2 and CO2 start do decrease after the initial activity peak just after lights off. After this initial drop in activity, the DR mice (D) do not display the second activity peak compared to the AL mice (A-C). This is well in line with the activity data from the DVC

		O2 ± SEM		CO2 ± SEM		RQ ± SEM	
Females - AL - 2.5months/pre-DR	Dark	104,3307	2,999342	100,6486	3,708087	96,74768	2,765865
	Light	74,47626	2,280354	71,38184	2,638987	96,16758	2,968827
Females - pre-DR - 2.5months	Dark	111,3115	4,546732	106,63	4,49062	96,07697	2,587222
	Light	83,94076	4,078802	77,87558	3,822566	92,9451	2,725426
Females - AL - 12months	Dark	80,20	4,013949	69,03	4,272577	85,89	3,160341
	Light	66,94	3,094776	56,84	3,521825	84,64	3,260761
Females - DR - 12months	Dark	78,31	4,813222	62,71	3,979616	81,27	2,711317
	Light	87,63	7,167414	76,14	6,871247	86,76	3,911898
Males - AL - 2.5months/pre-DR	Dark	77,54219	2,019677	75,73907	2,334013	97,75806	2,190714
	Light	60,57796	1,762327	56,68885	1,559684	94,35148	4,311865
Males - pre-DR - 2.5months	Dark	80,59833	3,03774	74,34856	2,613172	92,88266	3,161637
	Light	68,27275	2,370121	64,65191	2,58623	91,5163	3,242816
Males - AL - 5months	Dark	61,66243	4,974381	56,39215	4,397976	88,11766	6,560389
	Light	52,97107	4,043269	47,02143	3,52518	85,68874	6,379798
Males - DR - 5months	Dark	64,78881	6,930282	56,51756	6,067422	83,94145	5,304358
	Light	71,28197	9,652113	64,82775	8,188175	88,84857	6,82892
Males - AL - 22months	Dark	58,19302	5,129712	54,52419	4,815821	89,55568	7,153094
	Light	51,09572	4,438221	47,05832	4,186009	87,36992	6,854721
Males - DR - 22months	Dark	63,63875	6,444977	53,21459	4,310341	84,98511	4,461572
	Light	72,39955	11,9245	61,18149	8,070534	88,35579	7,373726

Table 2 Indirect calorimetry measures O2 consumption (ml/(min*kg), CO2 production (ml/min*kg) and the respiratory quotient (RQ) in percent. Mean values ± standard error of the mean, presented for each group (male and female mice, dietary restricted and ad libitum fed, before and after introducing diet regime and divided between daytime and nighttime).

Glucose tolerance test

AL mice are slower in clearing blood glucose after the i.p. injection. At time point 60 minutes after the injection, DR mice are already back to baseline level, while the AL mice need another 60 minute to clear the blood glucose.

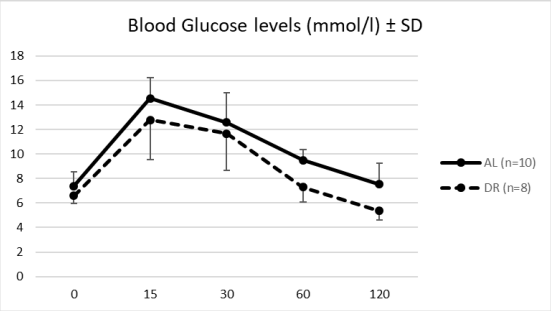

Fig 3. Clearance of blood glucose tested with intraperitoneal glucose tolerance test in 22 months old males, AL fed and dietary restricted (DR). The black line represents the AL fed mice and the dotted line represents the DR mice.

Home-cage activity, general patterns

The heat map in figure 4 very well illustrates the general activity pattern with higher activity daily during lights off and around the time for lights on in the holding room, as well as weekly immediately after cage change. During daytime, activity is low. The boxplots are showing weekly global activations divided into lights off (fig 4B) and lights on (4C). They confirm the activity during lights off but also displays a variation over time which seems to repeat in regular cycles. There is also a decline in nighttime activity levels with age, which is significant (see study II)

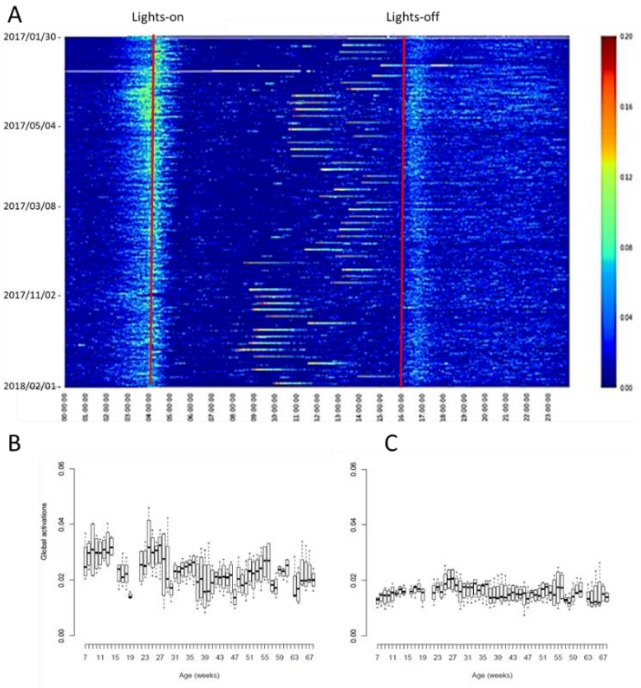

Fig 4 (A) Heatmap covering one year starting from the top. Each row respresents one day and starts at midnight to the left. The two red vertical lines indicate time for lights on and lights off.

B-C shows weekly global activations divided into night and day time.

In figure 5 we plotted the same cohort (the first cohort of males, see table 1) and the same time period split into daily, 12 hours aggregated nighttime activity. The rhythmicity of periods with higher and lower activity is even more evident, although there is a variation between cages.

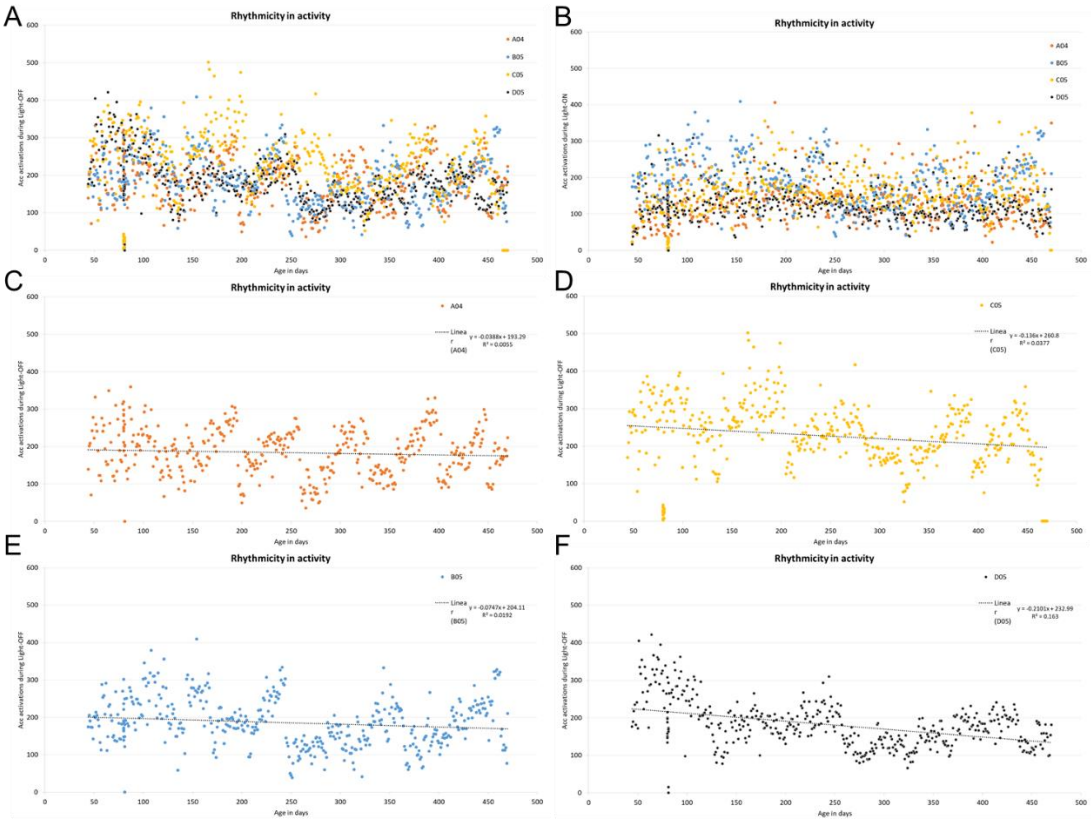

Fig 5. 12 hour aggregated activations, plotted each day instead of week first cohort of males). A represents nighttime data and all four cages and B is daytime. In C-F, each cage is plotted individually (nighttime)

Mice on dietary restriction, which were fed during the lights on period (10am – 2pm) completely switch their pattern and becomes highly active around time for feeding and very low in activity during night and around time for lights on in the holding room (figure 6)

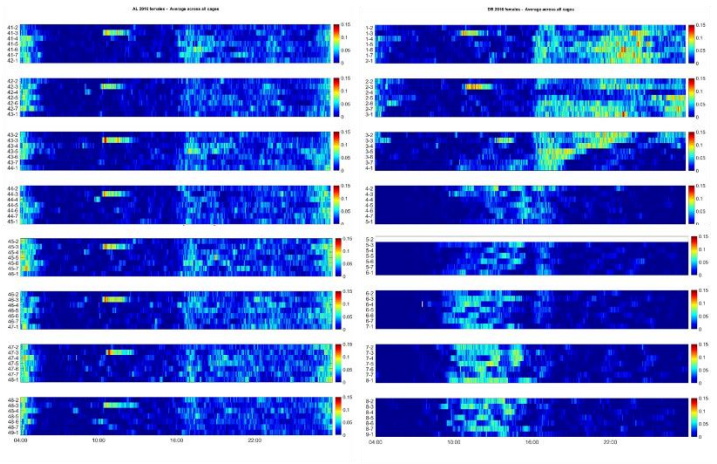

Figure 6 The left panel shows average activity in 3 cages of ad libitum fed, young female mice over 8 weeks. The right panel shows the same number of cages of females that were subjected to dietary restriction (during week number 3 from the top). Only a few days after the introduction of DR, the activity pattern is completely switched.

Difference in activity levels between weekdays and over time

Females monitored over a shorter time (10-15 weeks) showed significant variation in day-time activity between weekdays ($p<0.001$) and also between weeks at each of the three sites ($p<0.02$-0.001, see study I).

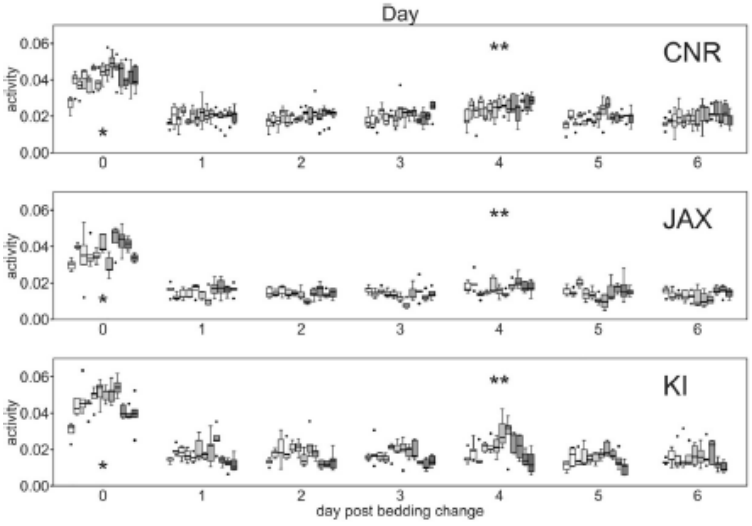

Fig 8. Daytime activity of the three female cohorts at the three different sites in the multi-center study. Global activity is plotted as mean across 5 cages for each week and for each day of the cage change cycle. The asterisk on day 0 indicates day of cage change and the two asterisks indicate the day of weighing.

The males monitored in study I also showed a significant variation in activity between weekdays ($p<0.001$) but not between weeks. In line with these results, the same group of males analyzed over longer time display difference in activity levels (both night, day)

between weekdays but not between weeks. This is also true for the other two cohorts of males and females (see study II).

Responses to procedures and events.

Cage change

Response to cage change was analyzed starting from the minute the cage was re-inserted into the rack, and the following 5 hours. We excluded all the cage changing procedures that occurred less than 4 hours before lights off.

In study I, cage change response in female cages were averaged and we compared peak response, average response activity as well as response duration between weeks. found significant variations in response between weeks, both within sites ($p<0.01$-0.001) and also between sites.

In the second study, we compared the response to cage change over a longer time in a different cohort of females (between ages 20-50 weeks) and found no significant variations in peak amplitude, response duration or average response activity between weeks.

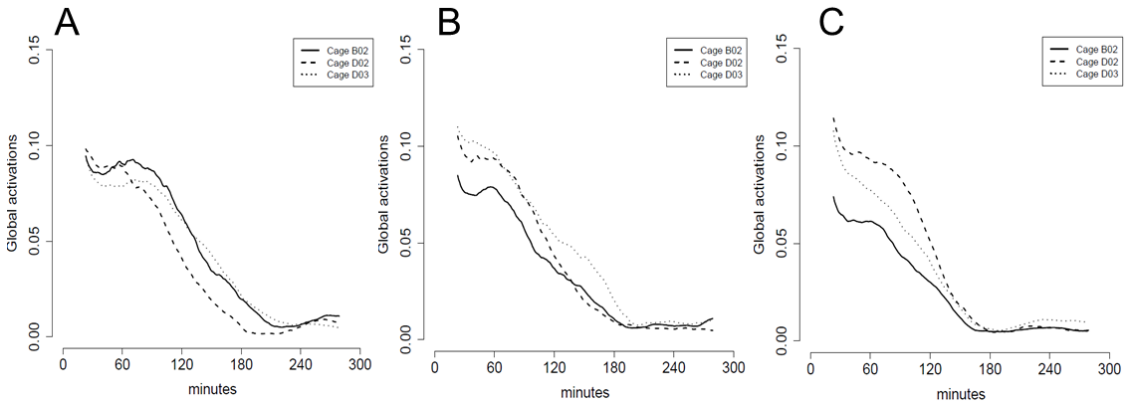

Fig 9 Cage change response analyzed in cages with ad libitum fed females from study II. The three plots represent the same cages but different ages; (A) 22-27 weeks, (B) 30-49 weeks and (C) 50-62 weeks.

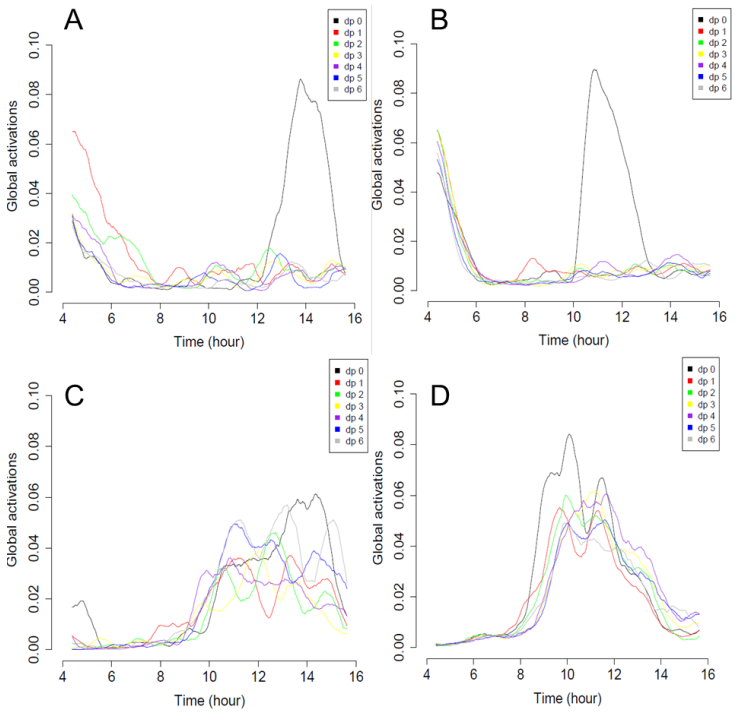

Fig 10 Daytime activity averaged in female cages and plotted separate for each day in the cage change cycle. The high peaks in the upper panel represents the response on cage change day in AL mice aged <20 weeks (A) and >50 weeks (B). The response in DR cages is not as clear, as it co-insides with the daily feeding.

Lights on

When analyzing response to lights on, we use 180 minutes before and 180 minutes after time for lights on and average across cages and weeks. Plotted in figure 11 is the response for each day of the cage change cycle in female and male mice from study II. Once the DR regime is introduced, lights on response is completely suppressed. In the AL fed mice, we found significant variations in lights on response between weekdays. It seems as the cage change

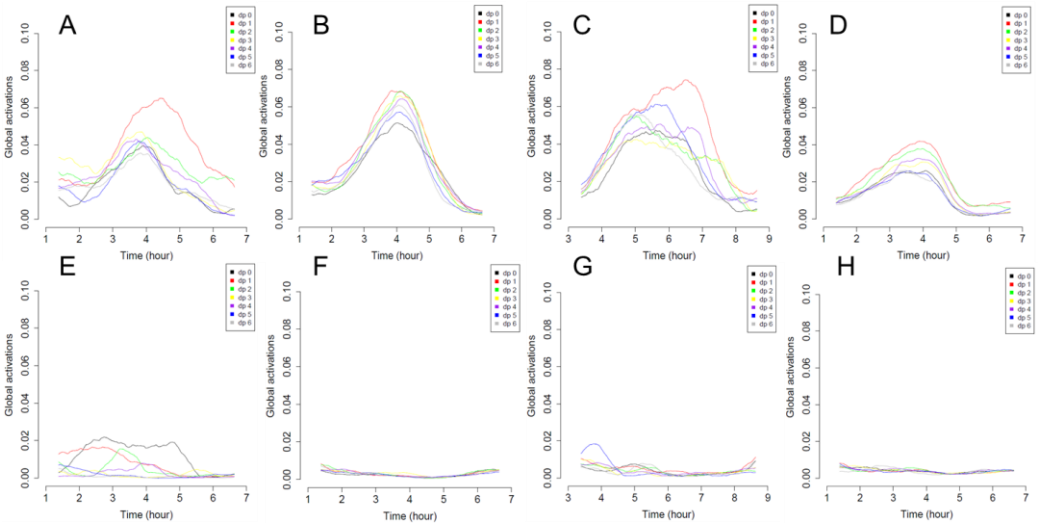

Fig. 11 Response to light-on which occurs at 4 AM (abscissa) for each weekday (colour coded) in young adult and middle aged AL fed females (A, B; grp3) and AL fed males (C, D; grp 2). Response is averaged across cages and weeks. In E-H the corresponding data for the cages with mice on DR in respective cohort. Once the DR regime has been established the response to lights-on is completely suppressed. In AL animals we confirm our earlier observation that that lights-on is anticipated and starts 1-2h before the lights actually are turned on.

The difference between diet groups was highly significant ($p<1^{-5}$ to 1^{-49}). In AL fed animals the response (peak and average activity) co-varied significantly with weekday (subplot factor) in all three cohorts ($p<1^{-2}$ to 1^{-6}).

Biorhythms

Figures 4, 5and 6 all show that there are re-occurring variations in activity level son top of the age-associated over-all decrease in both AL and DR mice. High-frequency oscillations as diurnal activity rhythm, week-to-week variations (see above and Paper II) have a higher amplitude in female than male mice. In addition, we discovered an oscillation with a frequency about 40 days (Fig 5) being most conspicuous during night time and with a considerable amplitude between high's and lows. Functional data analysis (Fig. 8 in Paper II) revealed rhythmic re-occurring episodes of high and low activity centred on the age-associated over-all decline in activity having a periodicity of 4-6 weeks. These oscillations were evident in both male and female cohorts and, furthermore, both during lights-off and lights-on (see Fig. 8 Paper II). Because day-time activity is low the alterations then have a small magnitude in both sexes.

4 CONCLUSIONS

Automated recording of laboratory animal's home cage behavior is receiving increasing attention since such non-intruding surveillance will aid in the unbiased understanding of animal cage behavior potentially improving animal experimental reproducibility.

The two studies of this thesis show that home cage monitoring is scalable and run in real time, providing complementary information for animal welfare measures, experimental design and phenotype characterization.

The recorded data allowed us to characterize floor activity recorded non-intrusively from group held C57Bl/6 mice of both sexes housed in standard IVCs 24/7 for extended period of time (up to ~700 days). Oscillation of activity occurred on daily basis (day and night), as a variance in activity across weeks (mainly observed among female mice) and as a seasonal-like oscillation with high's and low's and a periodicity of about 35-40 days in both sexes. Across the extended observation period we could also extract a small but highly significant overall decrease in activity between young adulthood (17 weeks of age) and middle age (>50 weeks of age). The recordings also suggest that acclimatization towards a harmonized pattern of rhythms may take 30-60 days following re-location and re-housing at a new holding quarter.

We also documented impact on mouse activity that standard animal handling procedures have, e.g. cage-changes, and show that such procedures are stressors impacting in-cage activity. Moreover, that re-occurring events like lights-on triggers a behavioral response. All these variances in activity behaviour are likely to influence experimental results in a range of studies and should therefore be considered in study design to facilitate reproducibility and implementation of the 3Rs.

Calorie or dietary restriction (CR/DR) has for long been known to extend life- and health span in all species so far examined. More recent studies on the impact of DR on mouse behaviors suggests that several behavior capacities known to dissipate with age are preserved until more advanced age by DR. The major observation made here is that the feeding regime imposed on DR animals made the mice swop day and night rhythm of activity, a transition that took but a few days. Furthermore, DR completely suppressed the lights-on response. Our observations suggest that behaviors related to basic needs as feeding can swiftly bring about fundamental changes to behaviors considered imprinted in the species and, importantly, these changes to behaviors and feeding is tightly linked to a healthier and longer life.

Finally, it worth noting that the activity traces (Figs 14 and 18 in Paper II) for AL and DR males and females, are very similar to the recorded traces for O_2 consumption and CO_2 production recorded with indirect calorimetry (Fig. 2 in Paper II), suggesting that activations recorded with the DVC translates to physical work

5 ACKNOWLEDGEMENTS

I would like to mention and say thank you to a few other people involved in this work.

Main supervisor **Brun Ulfhake**, thank you for offering me this opportunity and for being so patient and generous.

Co-supervisors **Velmurugesan Arulampalam** and **Rochellys Diaz Heijtz**, for contribution, support and advice and nice chats.

External mentor, **Katrin Wellfelt** for keeping me focused. And many other things.

Collaborators at Tecniplast, Fabio Iannello, Mara Rigamonti and Giorgio Rosati

Colleagues at Wallenberg: Adam Lada, Awahan Reheman, Beatrice Lind, Catarina Oliveira, Chiara Zullian, James Mount, Elisabet Andersson, Olga Lund, Niklas Lilja, Pontus Anstedt, Josefine Rosén, Emelie From, Kajsa Noaksson, Eva Gustafsson, Ellinor Olsen. Jimmy Nilsson, Johan Engström, Dan, Linda, Daniel.

6 REFERENCES

1. Reardon, S. (2016). "A mouse's house may ruin experiments." Nature **530**(7590): 264.

2. Pound P, Bracken MB (2014) Is animal research sufficiently evidence based to be a cornerstone of biomedical research? BMJ 348: g3387

3. Cressed D (2015) UK funders demand strong statistics for animal studies. Nature 520: 271-272.

4. Richardson CA (2015) The power of automated behavioural homecage technologies in characterizing disease progression in laboratory mice: A review. Applied Animal Behaviour Science 163: 19-27.

5. de Visser L, van den Bos R, Spruijt BM (2005) Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res 160: 382-388.

6. Logan S, Owen D, Chen S, Chen WJ, Ungvari Z, et al. (2018) Simultaneous assessment of cognitive function, circadian rhythm, and spontaneous activity in aging mice. Geroscience 40: 123-137.

7. Tang X, Orchard SM, Sanford LD (2002) Home cage activity and behavioral performance in inbred and hybrid mice. Behav Brain Res 136: 555-569

8. Tang X, Sandford LD (2005) Home cage activity and activity-based measures of anxiety in 129P3/J, 129X1/SvJ and C57BL/6J mice. Physiol Behav 84: 105-115.

9. Loos M, Koopmans B, Aarts E, Maroteaux G, van der Sluis S, et al. (2014) Sheltering behavior and locomotor activity in 11 genetically divers common inbred mouse strains using home-cage monitoring.

10. Adamah-Biassi EB, Stephien I, Hudson RL, Dubocovich ML (2013) Automated video analysis system reveals distinct diurnal bihaviors in C57BL/6J and C3H/HeN mice. Behav Brain Res 243: 306-312

11. Bains RS, Cater HL, Sillito RR, Chartsias A, Sneddon D, et al. (2016) Analysis of individual mouse activity in group housed animals of different inbred strains using a novel automated home cage analysis system. Frontiers in Behavioral Neuroscience 10.

12. Bains RS, Wells S, Sillito RR, Armstrong JD, Cater HL, et al. (2018) Assessing mouse behavior throughout the light/dark cycle using automated in-cage analysis tools. J Neurosci Methods 300: 37-47.

13. Pernold K, Iannello F, Low BE, Rigamonit M, Rosati G, Scavizzi F, Wang J, Raspa M, Wiles MV, Ulfhake B (2019), Towards large scale automated cage monitoring – Diurnal rhythm and impact of interventions on in-cage activity of C57BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique. PLoS One 14(2): e0211063.

14. Lamberts SWJ, van den Beld, van der Lely AJ (1997), The endocrinology of aging. Science 278: 419-424.

15. Bowles J (2000). Shattered: Medawar's test tubes and their enduring legacy of chaos. Med Hypotheses 54(2): 326-339.

16. Kirkwood TB (2002). Evolution of ageing. Mech. Ageing Dev. 123: 737-745.

17. Kirkwood TB and Shanley DP (2005). Food restriction, evolution and ageing. Mech Ageing Dev 126(9): 1011-1016.

18. Alberts P, Johansson BG, McArthur RA (2006), Characterization of energy expenditure in rodents by indirect calorimetry. Curr Protoc Neurosci, Chapter):Unit).23D

19. Alberts P, Johansson BG, McArthur RA (2005), Measurement and characterization of energy expenditure as a tool in the development of drugs for metabolic diseases, such as obesity and diabetes. Curr Protoc Pharmacol. Chapter 5:Unit5.39

20. Fahlström A, Zeberg H, Ulfhake B (2012), Changes in behaviors of male C57BL/6J mice across adult life span and effects of dietary restriction. Age (Dordr) 34(6): 1435-1452.

21. Altun M, Bergman E, Edström E, Johnson H, Ulfhake B (2007), Behavioral impairments of the aging rat. Physiol Behav 92(5): 911-923